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# Combining reflective mulch and host plant resistance for sweetpotato whitefly (Hemiptera: Aleyrodidae) management in watermelon

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ABSTRACT

A study was conducted to evaluate the use of reflective mulch and host plant resistance for the management of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), in watermelon [*Citrullus lanatus* var. *lanatus* (Thunberg) Matsum & Nakai]. Whitefly abundance data were collected under both greenhouse (caged and uncaged) and field conditions. Consistently, a reflective mulch (also called silver or metallic) treatment resulted in a lower incidence of adult whiteflies as compared with a standard black mulch treatment. Moreover, two whitefly resistant *Citrullus colocynthis* (L.) Schrad genotypes, which are wild relatives of cultivated watermelon, reduced whitefly populations as compared with standard watermelon. There was generally no interaction between the mulch and genotype variables. No effect of mulch color was observed on sticky card capture of *Delphastus catalinae* (Horn), a whitefly predator, or on capture of an *Eretmocerus* sp. whitefly parasitoid in caged trials, which suggests no adverse effect on these natural enemies when using reflective mulch. Overall whitefly populations were relatively low during four seasons of field trials (2006–2009). Results from this study suggest that a combination of using reflective mulch and host plant resistance can additively suppress whitefly infestations, which have particular importance in the fast-growing organic vegetable production industry.

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## 1. Introduction

Watermelon [*Citrullus lanatus* var. *lanatus* (Thunberg) Matsum & Nakai] is an important vegetable crop. However, watermelon is susceptible to numerous diseases and pests. The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), attacks watermelon and a wide range of other plant species and on a global scale. In addition to injuries from direct feeding, problems from this pest are intensified because it vectors over 100 plant viruses (Jones, 2003). Watermelon is among the many crops that are affected by whitefly-vectored viruses, especially in the southeastern region of the U.S. (Adkins et al., 2007; Akad et al., 2008; Polston et al., 2008). Although the application of insecticides is currently the most common method of whitefly control in watermelon, other strategies including cultural control and use of pest resistant crops may provide useful sustainable approaches in whitefly management schemes.

Several studies have demonstrated a reduction in infestation by insect pests and incidence of insect-vectored viral infection in vegetable crops by the use of reflective mulch (e.g., Wolfenbarger and Moore, 1968; Kring and Schuster, 1992; Smith et al., 2000; Stapleton and Summers, 2002; Summers et al., 2004; Frank and Liburd, 2005; Kousik et al., 2008; Nyoike et al., 2008). The primary targeted insects in reflective mulch studies have been virus vectors such as aphids, thrips and whiteflies. The previous research included several studies evaluating reflective mulch in cucurbit crops. For example, Nyoike et al. (2008) reported on a reduction in whitefly populations and a reduction in whitefly-vectored virus (*Cucurbit leaf crumple virus*) incidence in plots of zucchini squash (*Cucurbita pepo* L.) when reflective mulch was compared with white mulch. However, there have been few reports on reflective mulch studies on watermelon. McLean et al. (1982) demonstrated the use of reflective mulch in watermelon in Australia to reduce the incidence of *Watermelon mosaic virus* which is vectored by aphids.

Several PIs (Plant Introductions) of *Citrullus* have been identified that have resistance to the B-biotype sweetpotato whitefly, spider mites and broad mites (Simmons and Levi, 2002a,b; Lopez et al., 2005; Kousik et al., 2007). Plant resistance coupled with reflective mulch or other strategies may offer alternatives to relying solely on insecticide applications for the management of whiteflies and incidence of whitefly-associated viruses in watermelon production. The objective of this study was to assess the effectiveness of reflective mulch and host plant resistance for managing whiteflies in watermelon.

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## 2. Materials and methods

### 2.1. Source of insects

Insects used in greenhouse caged experiments were from a greenhouse colony maintained at the USDA-ARS, U.S. Vegetable Laboratory, in Charleston, S.C., USA. Except where noted otherwise, the whiteflies (B-biotype *B. tabaci*) used in the experiments had been maintained (Simmons, 1994) on assorted vegetable crops since 1992. The infestation of open greenhouse experimental plants was established by adult *B. tabaci* that had inadvertently invaded greenhouse cucurbit plants. Adults of *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae), a predator, were from a research colony. They were purchased commercially in 1999 and have since been maintained on a greenhouse colony of *B. tabaci*. Adults of an endemic *Eretmocerus* sp. (Hymenoptera: Aphelenidae) were from a greenhouse colony of *B. tabaci* which had been parasitized by a natural population of the parasitoid.

### 2.2. Greenhouse cage experiments

Two caged experiments were conducted on greenhouse benches. The first caged experiment was conducted using a dual-choice test for comparison of whitefly capture between 0.6 mil film of black and reflective (also called silver or metallic; metalized low density polyethylene, 100 gauge, 2.0 OD) mulches (Intergrow, Clearwater, Fla., USA). The reflective mulch has a 0.64 measured reflectance ($\rho$) and a 0.36 calculated near normal emitance ($\epsilon$) (Intergrow). Cages were constructed (45 cm wide $\times$ 45 cm long $\times$ 46 cm high) of Plexiglass. A 1-cm diameter hole in the center of the top of the cage allowed the insertion of insects. A Petri dish (10 cm diameter) was suspended below the hole to disrupt the insects from going directly to the bottom of the cage during their release. A Plexiglass side door allowed further access into the cage. Within each cage, half of the floor was covered with a rectangular strip of black mulch and the other half was covered with reflective mulch. One 5.1 $\times$ 5.1 cm$^2$ yellow sticky card was placed horizontally in the middle of each strip of mulch. Capture of *B. tabaci* was reported to be greater for horizontally placed cards than for vertically placed cards (Gerling and Horowitz, 1984; Lynch and Simmons, 1993). Three cages were utilized, and 100 adult whiteflies were released from a pipette through the ceiling entrance hole into each cage. The experiment was run from 2 h before to 2 h after solar noon. Four hours after the release, the number of whiteflies captured on each sticky card was counted with the aid of a - dissecting microscope. The experiment was repeated nine times for a total of 2700 insects tested. In addition, the experiment was conducted using 50 adult *D. catalinae* beetles, and this test was repeated nine times for a total of 1350 insects. The experiment was also conducted using 100 adult *Eretmocerus* sp. parasitoids. This test was repeated five times for a total of 1500 insects tested.

A second cage experiment in the greenhouse was set up using no-choice tests on watermelon seedlings. The cages were 0.61 m $\times$ 0.61 m $\times$ 0.61 m and were constructed with 70 mesh chiffon screening with aluminum frames and bottom (BioQuip® Products, Rancho Dominguez, Calif., USA). The floor of each cage was lined with either black or reflective mulch. One first true-leaf stage 'Sugar Baby' watermelon seedling was used in the center of each cage. Each plant was in a seedling tray; the tray was below the mulch while the plant protruded upward through a slit. Within each cage, 100 adult whiteflies were released 1 h before solar noon. The number of adult whiteflies on the plants was counted after 2 h, and the number of eggs deposited on each plant was determined with the aid of a microscope. Three cages of each mulch treatment were utilized for each trial. This experiment was conducted five times for a total of 1,500 adult whiteflies used per mulch treatment.

### 2.3. Greenhouse open choice experiment

A randomized split block design greenhouse choice test experiment was conducted using four germplasm entries and treatments of black and reflective mulches. Plants were established in 15.24 cm diameter (1500 ml) pots in Metro-Mix 360 potting soil (Sun Gro Horticulture, Seba Beach, Canada) and were grown to the 2–3 leaf stage. Five pots of each germplasm were set up for each treatment on a greenhouse bench. Pots were set up in a row on each side of the bench. The distance between pots was 36 cm. Five replicates of germplasm were set up for the experiment using four genotypes [two *C. lanatus* var *lanatus* ('Crimson Sweet' and 'Mickylee') and two *C. colocynthis* (PI 386015 and PI 386024)]. All plant accessions used in all experiments herein were obtained from the *Citrullus* collection of the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia, USA. Genotypes were randomly assigned within each split plot. Mulch color consisted of each main plot. A 5.1 $\times$ 5.1 cm$^2$ sticky card was placed horizontally on the mulch near the center plant of each genotype. At weekly intervals, the numbers of whiteflies on the sticky cards were counted and the cards were replaced with new cards. The 3$^{rd}$ leaf from the terminal was collected and numbers of eggs and nymphs of *B. tabaci* were recorded with the aid of a microscope. The experiment was conducted twice, in 2006 and 2007, while only two genotypes (Mickylee and PI 386015) were evaluated in 2007.

### 2.4. Field experiment

Field trials were conducted during four summers (2006, 2007, 2008 and 2009). A split–split plot design was utilized for the experiment during the first three years. The experimental factors consisted of four germplasm entries, two mulch treatments and two insecticide treatments (consisting of an insecticide and an untreated check). Four blocks were set up with the two mulch treatments as the main plots, insecticide (a soil drench application of imidacloprid at 560 ml/ha) as the subplot and germplasm as the sub–subplot. The germplasm treatments were two *C. lanatus* var *lanatus* ('Crimson Sweet' and PI 248178) and two *C. colocynthis* (PI 386024 and PI 386015). The first two are susceptible and the latter two are resistant to whiteflies (Simmons and Levi, 2002a, 2002b). Seeds of each genotype were germinated in the greenhouse, and five seedlings at the 2–4 leaf stage (4 wk after seeding) were transplanted in each sub-subplot. However, PI 248178 was replaced with Mickeylee and PI 386024 was not included in the field trials in 2007 and 2008. Mulches were black or reflective (as described above). No post-emergence pesticide was used in any plot, except imidacloprid (0.01% Admire®) was used in the insecticide plots within a day of transplanting. In 2006 and 2007, single row plots were established with 10 plants per plot and 46 cm between plants. Each plot was 4.6 m long and there were 4.6 m between plots. There were 6 m between row beds. The beds were 20 cm high, and the mulch covered a width of 0.97 m of the beds. In 2008, 2 row plots as described above were used for each treatment. Transplant dates were on 13 July, 11 July and 23 July in 2006, 2007 and 2008, respectively. Plant genotypes were randomized and replicated once within each insecticide treatment. One week after the transplant, yellow sticky cards (16 cm$^2$) were placed horizontally, 15 cm above row beds and located between two plants in the middle of each plot of each genotype. As described for the open greenhouse experiment, the number of whiteflies per sticky card was recorded and the cards were replaced weekly.

**Table 1**
Mean number of adult beetles (*D. catalinae*), parasitoids (*Eretmocerus* sp.) and whiteflies (*B. tabaci*) captured on sticky cards in dual-choice tests with cages lined with two mulches in a greenhouse 4 h after the release of the whiteflies.

| Insect | N[a] | No. insects tested[b] | Mean number of insects captured on mulch | | |
|---|---|---|---|---|---|
| | | | Black (SEM) | Reflective (SEM) | $P > t$ ($t$ value) |
| Beetles | 9 | 1,350 | 1.81 (0.99) | 1.96 (0.52) | 0.842 (−0.20) |
| Parasitoids | 5 | 1,500 | 2.40 (0.58) | 1.20 (0.63) | 0.26 (1.18) |
| Whiteflies | 9 | 2,700 | 43.07 (3.52) | 10.07 (1.66) | <0.0001 (8.49) |

Probabilities of different means in a row based on Paired Comparison $t$ Test (SAS, 2003).
[a] $N$ denotes the number of times this experiment was repeated.
[b] Total number of insects used for this experiment in all replications.

**Table 3**
Mean number of whiteflies (*B. tabaci*) captured on sticky cards and number of eggs per leaf sample across genotypes of *Citrullus* spp. on black and reflective mulches in an open greenhouse.

| Year | Stage | $N$ | Mean number of whiteflies (SEM) | |
|---|---|---|---|---|
| | | | Black mulch | Reflective mulch |
| 2006 | Adult | 100 | 129.8 (18.7)a | 59.01 (4.8)b |
| | Egg | 27 | 20.4 (9.4)a | 8.9 (2.9)a |
| 2007 | Adult | 100 | 187.8 (9.9)a | 93.1 (6.8)a |

Means in a row followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls Test (SAS, 2003).

Although yellow sticky cards were used in all aforementioned experiments, an influence of the yellow color of the cards on the attraction of the whiteflies to the plants was not ascertained. Thus, an additional experiment was conducted in 2009 to assay the mulch and germplasm treatments without the use of yellow sticky cards. A randomized split block was set up with mulch treatments (black and reflective) as the main plots and genotypes (Crimson Sweet, PI 386024 and PI 386015) as the subplots. Two plants of each genotype were used for each mulch treatment, and three blocks of treatments were set up. After the plants reached the 4–5 leaf stage, counts of adult whiteflies were made by gently turning over the leaves. Six times within 4 weeks, the number of adults on the 3rd and 4th leaves of each plant was recorded. The experiment was repeated twice.

### 2.5. Data analysis

For the dual-choice cage experiment, comparisons between treatment counts were made using the Paired Comparison $t$ test. For the other experiments, data were subjected to ANOVA using the Mixed Procedure Test before means were separated using the Student–Newman–Keuls Test (SAS, 2003). Significant differences were determined at $P < 0.05$.

## 3. Results and discussion

### 3.1. Greenhouse experiments

The greenhouse trials consistently supported fewer whiteflies on the reflective mulch than on black mulch treatment. In the dual-choice experiment without plants, four times as many whiteflies were captured on sticky cards in the treatment of the black versus the reflective mulch (Table 1). Mulch color did not have any significant influence on the capture of adults of the predator or parasitoid, *D. catalinae* and *Eretmocers* sp., respectively ($P > 0.06$) (Table 1). Thus, these results suggest that the use of reflective mulch may be compatible with natural enemies while lessening the population of whiteflies. At the end of each caged experiment, numerous live whiteflies, beetles and parasitoids were observed inside of the cages. Because no significant effect by the mulches was observed for the capture of the two natural enemies, no additional experiment was conducted with these two insects. The overall incidences of beetle and parasitoid capture on yellow sticky cards were low, which are consistent with results from previous studies on the capture of these two natural enemies as compared with the capture of *B. tabaci* (Hoelmer et al., 1998; Simmons, 1998, 2003; Qiu and Ren, 2006; Hoelmer and Simmons, 2008). Moreover, in the no-choice caged experiment with plants, the numbers of adult whiteflies and eggs found on watermelon seedlings were significantly lower for the reflective mulch treatment as compared with the black mulch treatment (Table 2).

In the uncaged open choice greenhouse experiment, across all germplasm treatments, the number of adult whiteflies was more than two times as great on traps in the black mulch treatment as compared with the reflective mulch treatment (Table 3). Moreover, whitefly egg counts tended to be elevated on plants in the black mulch treatment, but the egg counts were not significantly different from counts in the reflective mulch treatment (Table 3). In the greenhouse experiment, adult whitefly counts were consistently elevated on black mulch, but there was only a marginal genotype entry effect ($P = 0.06$, Table 4). In both years, whitefly capture was significantly affected by mulch color and week, with no significant interaction between these factors (Table 4). Whiteflies are well known for being affected by certain colors (Lloyd, 1921). No other interaction was detected among the sources of variations. Over time (week), adult capture was elevated earlier in both experiments as compared with later observations when vines covered much of the surface of the mulched experimental area around the plants.

**Table 2**
Mean number of whiteflies (*B. tabaci*) on leaves of 'Sugar Baby' watermelon seedlings in a no-choice test using cages lined with black and reflective mulches in a greenhouse after 2 h of release.

| Stage | No. of observations | Mean number of whiteflies (SEM) | |
|---|---|---|---|
| | | Black mulch | Reflective mulch |
| Adult | 27 | 10.26 (0.87)a | 1.41 (0.43)b |
| Eggs | 27 | 5.33 (1.07)a | 0.56 (0.25)b |

Means in a row followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls Test (SAS, 2003).

**Table 4**
ANOVA of number of whiteflies (*B. tabaci*) captured on sticky cards on reflective and black mulches across time for *Citrullus* germplasm in a greenhouse.

| Year | Source of variation[a] | $F$ | df | $P > F$ |
|---|---|---|---|---|
| 2006 | Color | 16.80 | 1 | 0.0001 |
| | Entry | 0.78 | 3 | 0.5072 |
| | Week | 11.92 | 4 | 0.0001 |
| | Color × Entry | 0.40 | 3 | 0.7543 |
| | Color × Week | 8.00 | 4 | 0.0001 |
| | Entry × Week | 0.23 | 12 | 0.9966 |
| | Color × Entry × Week | 0.18 | 12 | 0.9989 |
| 2007 | Color | 74.78 | 1 | 0.0001 |
| | Entry | 3.40 | 1 | 0.0667 |
| | Week | 11.08 | 4 | 0.0001 |
| | Color × Entry | 0.57 | 1 | 0.4513 |
| | Color × Week | 2.04 | 4 | 0.0902 |
| | Entry × Week | 0.67 | 4 | 0.6127 |
| | Color × Entry × Week | 0.25 | 4 | 0.9101 |

[a] Colors are black or reflective mulches; entries consisted of two cultivars and two PIs in 2006 and one cultivar and one PI in 2007. The analysis are according to the Mixed Procedure Test (SAS, 2003).

**Table 5**
Mean number of whiteflies (*B. tabaci*) captured on sticky cards on reflective and black mulches across insecticide and *Citrullus* germplasm in a field.

| Mulch color | Mean number of adult whiteflies per week (SEM) | | |
|---|---|---|---|
| | Year 2006 | Year 2007 | Year 2008 |
| Black | 19.9 (0.8)a | 6.9 (0.5)a | 12.9 (1.4)a |
| Reflective | 18.1 (0.9)a | 4.1 (0.8)b | 12.1 (1.4)a |

Means in a column by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls Test (SAS, 2003).

### 3.2. Field experiments

In the 2006–2008 field experiment, there was a consistent trend of a mulch effect, but the reflective mulch treatment only resulted in significant reduced whitefly capture during one of three years (Table 5). Plant genotype had a significant effect on whitefly capture during two of three years of the experiment (Table 6). Consistently fewer whiteflies were captured in plots of PI 386015. A mulch by plant genotype interaction was not detected during any of the three field seasons, and no significant effect was observed in response to the insecticide treatment. Thus, the contribution on reduced whitefly populations by reflective mulch and resistant genotype appears to be additive. In the field, the population of whiteflies collected was very low during each of the three years. Moreover, as the season progressed, the test plants began to cover more of the mulch which may have helped to diminish any effect of mulch color on the adult whitefly behavior.

Data from the 2009 experiment (when yellow sticky cards were excluded) were consistent with data from the other experiments, even though whitefly counts on leaves were very low. Reflective mulch significantly reduced whiteflies ($F = 11.14$, $df = 1$, $P < 0.0009$). The resistant germplasm supported lower number of adult whiteflies ($F = 52.61$, $df = 2$, $P < 0.0001$) as compared with the control treatments (Table 7). The two *C. colocynthis* PIs, which are wild relatives of the cultivated watermelon, had the least whitefly numbers (Table 7). This is the only test where an interaction was detected between mulch and germplasm treatments ($F = 9.34$, $df = 2$, $P < 0.0001$). The effect was synergistic on whitefly counts in plots with the reflective mulch and resistant genotypes providing the lowest whitefly count. Because whiteflies are highly attractive to yellow (Gerling and Horowitz, 1984), this experiment excluded any possible interaction based on whitefly attraction to the yellow cards. Frequent rainfall and many days of overcast skies existed during much of the experiment, and numerous adult parasitoids were observed in flight around the leaves. These factors may have had an adverse effect on whitefly population. Rainfall can negatively impact populations of *B. tabaci* (Basu, 1995). Furthermore, overcast days can reduce the amount of reflection from a surface, although a mulch effect was detected.

**Table 6**
Mean number of whiteflies (*B. tabaci*) captured on sticky cards among germplasm of *Citrullus* across mulch and insecticide treatments in a field.

| Entry | Mean number of adult whiteflies/sticky trap/week (SEM) | | |
|---|---|---|---|
| | Year 2006 | Year 2007 | Year 2008 |
| PI 248178 | 19.9 (1.4)a | – | – |
| PI 386024 | 19.3 (1.4)a | – | – |
| Crimson Sweet | 19.0 (1.1)a | 7.5 (1.0)a | 17.9 (2.2)a |
| Mickeylee | – | 5.8 (0.9)a | 14.5 (1.6)a |
| PI 386015 | 17.7 (1.1)a | 3.1 (0.4)b | 5.3 (0.7)b |

Means in a column followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls Test (SAS, 2003).

**Table 7**
Mean number of adult whiteflies (*B. tabaci*) on 3rd and 4th leaf from vine terminal for genotypes of *Citrullus* spp. across mulch treatments in field plots in 2009.

| Entry | N | Mean number of whiteflies (SEM | |
|---|---|---|---|
| | | Trial 1 | Trial 2 |
| Crimson Sweet | 72 | 2.72 (0.44)a | 1.25 (0.21)a |
| PI 386024 | 72 | 0.14 (0.10)b | 0.07 (0.03)b |
| PI 386015 | 72 | 0.35 (0.10)b | 0.10 (0.04)b |

Means in a column followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls Test (SAS, 2003). Within each trial, mulch treatments were significantly different ($P < 0.05$; means = 1.45 and 0.69 for Trial 1 black and reflective mulch, respectively and 0.65 and 0.21 for Trial 2 black and reflective mulch, respectively).

In conclusion, the data support the previously observed negative impact of reflective mulch on the population of some insect pests. Because no adverse impact was observed on two beneficial species (a coleopteran predator and a hymenopteran parasitoid), this suggests an added utility of using reflective mulch for sustainable watermelon production. In agreement with earlier reports (Simmons and Levi, 2002a,b), the field research consistently demonstrated whitefly resistance by PI 386015, although the effect was less discernable in the greenhouse research. Elucidation of alternatives to traditional insecticide applications will facilitate their use as whitefly management components in watermelon and other vegetable crops. Reflective mulch and plant resistance offers particular complementary potential to whitefly management in the fast-growing organic vegetable production industry. Any reduction in whitefly infestation may also have implications on the infection of viruses that are vectored by whiteflies on watermelon as has been observed by McLean et al. (1982).

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